Highly efficient dissociation of distinct adult rodent brain regions ensures high cell viability and recovery for successful downstream processing

Sandy Reiß, Jens Gaiser, Andreas Bosio, and Melanie Jungblut
Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

Introduction

Careful tissue dissociation and preparation of single-cell suspensions with high cell viability and a minimum of cell debris are prerequisites for reliable cellular analysis. In case of adult brain, sophisticated mechanical and enzymatic treatments are required to disaggregate the tightly connected neural cells successfully. The process becomes even more challenging when only small brain regions serve as starting material for the generation of single-cell suspensions, and when highest possible viability and recovery and well-preserved functionality are crucial for successful downstream processing.
In the past, we established elaborated technologies for the automated dissociation of adult rodent brain. These are based on mechanical dissociation using the gentleMACS™ Octo Dissociator with Heaters and an optimized enzymatic treatment, followed by an effective removal of debris and erythrocytes. The protocol was now further refined to enable the highly efficient generation of single-cell suspensions from small brain regions, e.g., hippocampus, cerebellum, olfactory bulb, or cortex with high viability and neural cell yield and a minimum of contaminating debris. Cells were then subjected to cell separation techniques that allowed for purification of neurons and astrocytes and subsequent cultivation of astrocytes.
In summary, we developed an optimized standardized technology that enables gentle and efficient dissociation of various distinct brain regions with high cell viability and preserved functionality for downstream processing.

Results

1 Automated dissociation of distinct adult brain regions and downstream processing

Brain regions such as hippocampus, cortex, and cerebellum were dissected from adult CD1 mouse brains (>P7) and dissociated using the Adult Brain Dissociation Kit, mouse and rat and the gentleMACS Octo Dissociator with Heaters. Debris and erythrocytes were subsequently removed to increase the percentage of intact neural cells. Neurons or astrocytes were then either isolated by magnetic cell separation (MACS® Technology) or by flow sorting using the microchip-based microfluidic flow sorter MACSQuant® Tyto®. Moreover, isolated cells were analyzed by flow cytometry and cultivated (fig. 1). The dissociation protocol yielded $1.03\times10^6\pm6\times10^5$ cells (n = 5) per cerebellum, whereas $6.3\times10^5\pm2.8\times10^5$ cells were obtained from one cortical hemisphere (n = 4), $1.9\times10^5\pm9\times10^4$ cells from olfactory bulbs of one mouse (n = 4), and $3.3\times10^5\pm9.5\times10^4$ cells from two hippocampi (n = 9). Viability rates were between 70 and 80%, depending on the brain region (fig. 2).

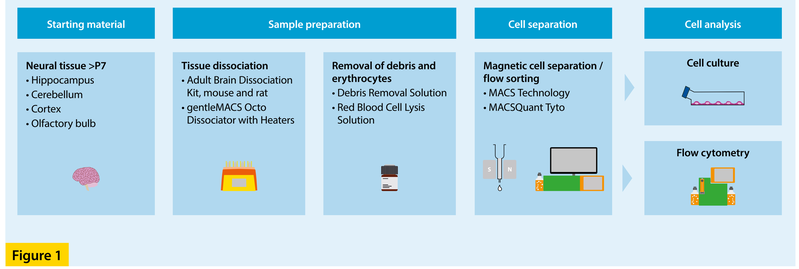

Figure 1

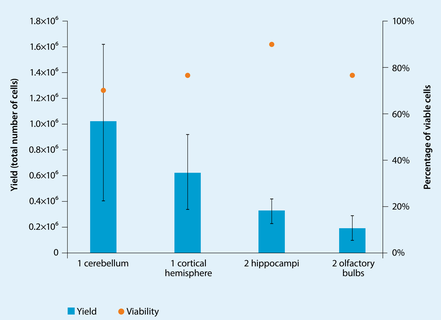

Figure 2

2 Identification of different neural cell types after dissociation of hippocampus and cortex

After dissociation of hippocampus and cortex, the different neural cell types were identified by flow cytometry on the MACSQuant Analyzer 10. The following antibodies were used to identify the cell types specified in parentheses: i) CD11b (microglial cells), ii) Anti-ACSA-2 (astrocytes), iii) CD140a, also known as PDGFRα, (oligodendrocyte precursor cells, OPCs), iv) Anti-O4 (oligodendrocytes). An antibody cocktail detecting non-neuronal cells enabled identification of neurons (fig. 3). Dissociated hippocampus tissue consisted of 24% microglia, 21% astrocytes, 18% OPCs, 42% oligodendrocytes, and 5% neurons, whereas the proportions in dissociated cortex were 26% microglia, 40% astrocytes, 9% OPCs, 33% oligodendrocytes, and 8% neurons (fig. 3).

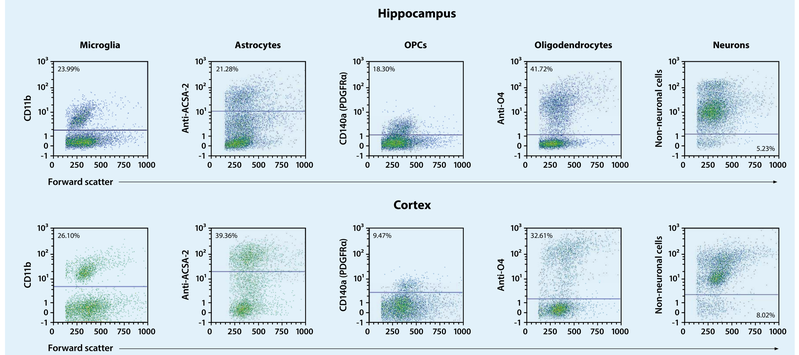

Figure 3

3 Isolation of neurons from distinct brain regions

Neurons were indirectly labeled with a non-neuronal cell–specific antibody cocktail and isolated from dissociated adult cerebellum, cortex, and olfactory bulb using the MACSQuant Tyto, a new, easy-to-use benchtop flow sorter (fig. 4A, top). This multiparameter cell sorting device uses a microchip-based technology for gentle cell isolation. The entire sorting procedure of this instrument occurs within the closed environment of the MACSQuant Tyto Cartridge (fig. 4A, bottom), thus enabling sterile processing. Unlike in conventional droplet sorters, cells do not experience high pressures, and no charge is applied, ensuring high cell viability and functionality. Sorted cell samples were analyzed by flow cytometry using the MACSQuant Analyzer 10. One cerebellum delivered 1.2×10^4 neurons with a purity of 80% and a viability of 97%, while one cortical hemisphere yielded 4.4×10^3 neurons (purity: 99%; viability: 95%), and two olfactory bulbs resulted in 4.8×10^3 neurons (purity: 99%; viability: 96%). Figure 4B shows a representative analysis of the fractions before and after sorting. Debris, doublets, and dead cells were excluded from the analysis.

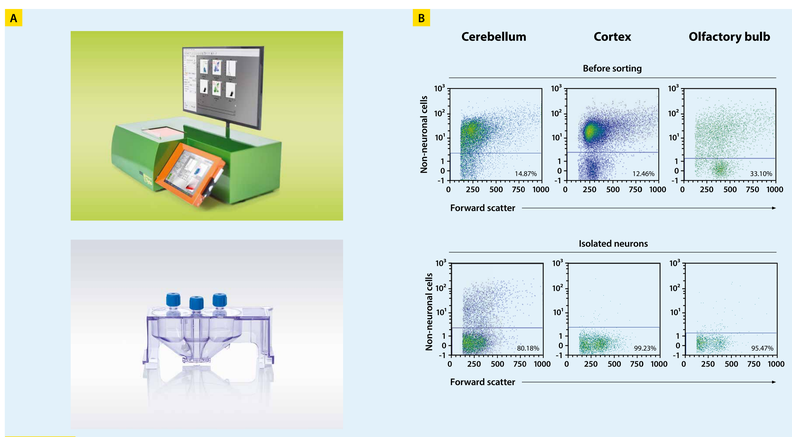

Figure 4

4 Isolation and cultivation of astrocytes from distinct brain regions

After dissociation of cerebellum, cortex, and hippocampus from adult mice, cells were labeled with MACS MicroBeads coupled to antibodies specific for the astrocyte marker ACSA-2 (astrocyte cell surface antigen-2) and isolated by MACS Technology (fig. 5A). Unseparated and enriched cells were stained with Anti-ACSA-2-PE for flow cytometry analysis (fig. 5B). Enriched astrocytes showed purities of 96 to 97%. A total of 1×10^5 living astrocytes were obtained from one cerebellum, whereas 1.25×10^5 viable astrocytes were isolated from one cortical hemisphere, and 9.7×10^4 astrocytes from two hippocampi of one adult mouse brain. Viability rates were between 70 and 87%.

Isolated astrocytes from cerebellum and hippocampus were cultivated in AstroMACS Medium, i.e., MACS Neuro Medium supplemented with MACS NeuroBrew-21 and AstroMACS Supplement, on PLL/laminin–coated 96-well glass bottom imaging plates. After 7 days, cells were fixed and subjected to immunocytochemical analysis using antibodies specific for GLAST and ACSA-2 (fig. 6A and B, upper row) or GLAST and GFAP (fig. 6A and B, lower row). Cells formed a dense layer of GLAST-positive astrocytes that were mostly ACSA-2-positive and displayed the characteristic morphology. Only very few astrocytes showed expression of GFAP.

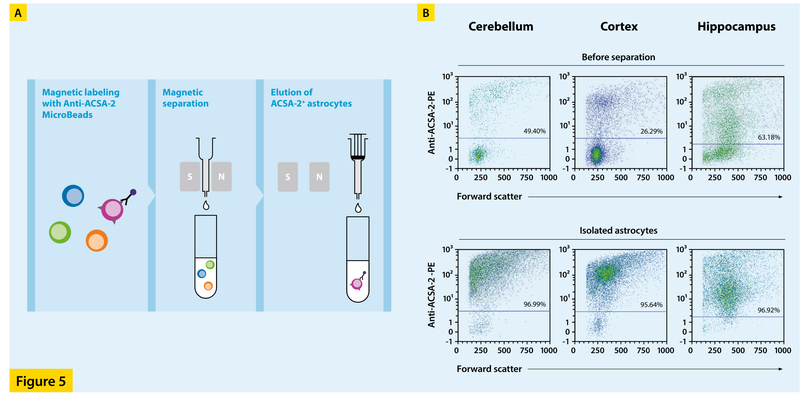

Figure 5

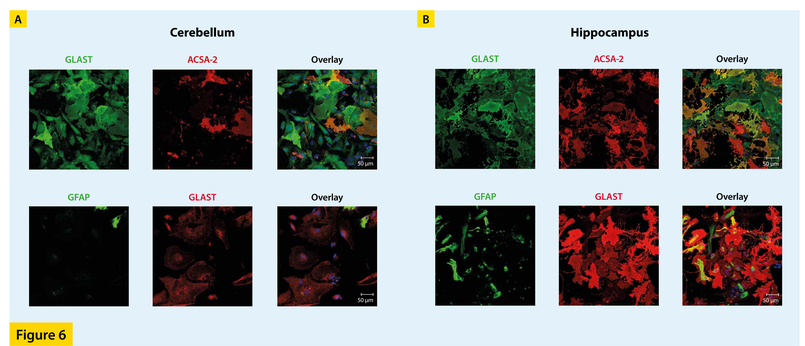

Figure 6

Conclusion

- We present a novel approach for the gentle automated and rapid dissociation of distinct regions of adult rodent brain tissue resulting in single-cell suspensions with high cell viability.
- The Adult Brain Dissociation Kit, mouse and rat enables the isolation of viable neural cells from small distinct brain regions.
- Tissue dissection and dissociation as well as isolation of neural cells can be performed in only 4 h.
- Gentle cell sorting on the MACSQuant Tyto allows enrichment of neurons from distinct brain regions to a purity of up to 99%. Cells are suitable for detailed downstream analysis.
- Highly pure astrocytes from distinct brain regions can be cultivated and applied to further studies.